Serb. Astron. J. № 176 (2008), 1 - 4

EVIDENCE OF MOLECULAR ADAPTATION TO EXTREME ENVIRONMENTS AND APPLICABILITY TO SPACE ENVIRONMENTS

M. Filipović[1], S. Ognjanović[2] and M. Ognjanović[2]

[1] *University of Western Sydney, Locked Bag 1797, Penrith South DC, NSW 1797, Australia*

[2] *University of Minnesota, MMC 715, 1-185 Moos 420 Delaware Street SE Minneapolis, MN 55455, USA*

(Received: 20 February, 2008; Accepted: 1 April, 2008)

SUMMARY: This is initial investigation of a gene signatures responsible for adapting microscopic life to the life in extreme Earth environments. Here, we present a preliminary results on identification of the clusters of orthologous groups (COGs) common to several hyperthermophiles and exclusion of those common to a mesophile (non-hyperthermophile): *Escherichia coli (E. coli K12)*, **will yield a group of proteins possibly involved in adaptation to life under extreme temperatures. Comparative genome analyses represent a powerful tool in discovery of novel genes responsible for adaptation to specific extreme environments. Methanogens stand out as the only group of organisms that have species capable of growth at 0°C (***Metarhizium frigidum (M. frigidum)*** and ***Methanococcoides burtonii (M. burtonii)***) and 110°C (***Methanopyrus kandleri (M. kandleri)***). Although, not all the components of heat adaptation can be attributed to novel genes, the** *chaperones* **known as heat shock proteins stabilize the enzymes under elevated temperature. However, highly conserved** *chaperons* **found in bacteria and eukaryots are not present in hyperthermophilic Archea, rather, they have a unique** *chaperone TF55*. **Our aim was to use software which we specifically developed for extremophile genome comparative analyses in order to search for additional novel genes involved in hyperthermophile adaptation. The following hyperthermophile genomes incorporated in this software were used for these studies:** *Methanocaldococcus jannaschii (M. jannaschii), M. kandleri, Archaeoglobus fulgidus (A. fulgidus)* **and three species of** *Pyrococcus*. **Common genes were annotated and grouped according to their roles in cellular processes when such information was available and proteins not previously implicated in the heat-adaptation of hyperthermophiles were identified. Additional experimental data is needed in order to learn more about these proteins. To address a non-gene based components of thermal adaptation, all sequenced extremophiles were analysed for their GC contents and aminoacid hydrophobicity. Finally, we develop a prediction model for optimal growth temperature.**

Key words. Astrobiology – Extremophiles

1. INTRODUCTION

Understanding life in extreme environments on Earth can tell us a great deal about the potential for life in similar environments on other celestial object such as planets, satellites, comets and asteroids. Understanding the limits of life as we know can also help determine what makes a planet habitable.

Astrobiology has developed as a new field, devoted to the scientific study of life in the universe – its origin, distribution, evolution and future. This multidisciplinary field brings together the physical and biological sciences to address some of the most fundamental questions of the natural world: the origin of life, evolution of habitable worlds and adaptations of terrestrial life required for potential survival beyond our home planet.

We now realise that the origin and evolution of life itself cannot be fully understood unless viewed from a larger perspective than just our own planet – Earth. Biologists are working with astronomers to describe the formation of life's biochemical precursors, and to discover new potentially habitable planets, while collaborations with computer scientist, geologists, paleontologists, evolutionary biologists, climatologists and planetary scientists help studies of other aspects of life limits.

Our intention is to investigate a gene signature responsible for adapting microscopic life forms to the life in extreme Earth environments with the goals to:

(i) test if computationally identified genes are expressed in a group of psychrophiles

(ii) characterize computationally identified proteins and their functions

(iii) to investigate whether such genes can be used to modify organisms which can be used for terraforming suitable planets.

(iv) predict what is the range of environmental conditions on the other planets and solar bodies that allows the existence of the basic life forms

Revelations about extremophiles have invigorated the field of astrobiology (Feller & Gerday 2003). In recent years, the field has also been stimulated by the discoveries of apparently biogenically derived methane on Mars (Onstott et al. 2006), the knowledge that methanogens exist and are active in the cold, and that methanogens can grow and metabolize in Martian-soil stimulant (Cavicchioli 2002). Other exciting findings have been the discoveries of live microorganisms in ice cores taken from sea ice (Price 2007; Tung 2005) and the presence of water in cold environments on Mars (ice sheets and permafrost), Mercury and Europa (sediments deep beneath the icy crust (Christner et al. 2001)). Cryopreserved micro-organisms can remain viable (in a deep anabiotic state) for millions of years frozen in permafrost and ice. *Psychrophiles*, cold loving bacteria, proliferate at temperatures 0°–10°C, metabolize in snow at ice at –20°C, are predicted to metabolize at –40°C and can survive at –45°C (Goodchild et al. 2004; Feller & Gerday 2003; Margesin 1999; Price & Sowers 2004; Sounders et al. 2003; Siddiqui & Cavicchioli 2006; Wagner et al. 2005). It is estimated that more than 80% of biosphere is permanently below 5°C (Cavicchioli et al. 2000).

All components of *psychrophiles*, must be adapted to cold to enable an overall level of cellular function that is sufficient for growth and survival. Cold adaptations in bacteria affects most structural and functional components of the cell; ranging from the outer membranes (lipid composition) to the inner cellular machines (ribosomes), protein translation processes, enzymes and nucleic acids (tRNA) (Feller & Gerday 2003; Margesin & Schinner 1999). Often there are other life-limiting factors present in these cold environments, such as high pressure (deep sea), high levels of UV irradiation (snow and ice cap communities), aridity (Antarctic cryptoendoliths), low light (cave-dwelling). Despite some advances in understanding molecular adaptations to cold (D'Amico, et al. 2002; Demming 2002; Feller & Gerday 2003), these adaptations remain poorly understood. The studies have shown that psychrophilic metabolic activities may contribute to weathering processes and carbon/nutrient cycling (Skidmore et al. 2000; 2005; Hearn 2003) and that these organisms may be utilized for biotechnological, agricultural, industrial purposes, as well as potential bioremediation in cold regions (Cavicchioli 2002).

2. AIMS AND APPROACH

A recent study of NASA Ames Institute on Atacama Desert (the driest desert on Earth) showed that life on this planet is limited by the presence of water (Navaro-Gonzales et al. 2003). Understanding the limits of life on this planet as well as specific adaptations required for survival in extreme environments represents an important contribution to the efforts of searching for life or sustaining it in space.

Our aims are to elucidate the genetic mechanisms underlying the adaptations to specific extreme environments and the effect of two physical parameters (pressure and temperature) on adaptation and limitation of life.

The choice of extremophiles to be studied is based on the hypothesis that there is water under thick layers of ice on Mars, Mercury and Europa, concluding that such a water environment would be under high hydrostatic pressure, high temperature (at the places of hydrothermal vents) or low temperature, therefore we chose to study piezophiles, hyperthermophiles and psychrophiles, while extreme aquatic habitats of hydrothermal vents of Lohihi vol-

cano (Hawaii, USA) and deep ocean will serve as earth analogues of such space environments.

We plan to develop a database of environments found to date in space, as well as computer models of hypothetical environments that could exist in space. We would then create a computer application interfacing between the extremophile properties and the modeled and existing environments in order to investigate what kind of organisms can be expected or cultivated on other planets.

3. METHOD

Understanding constrains on microbial populations in extreme environments is of great interest in the context of Earth analogs for possible extraterrestrial habitats. The application of DNA microarray technology to studies of life in extreme environments offers an outstanding opportunity for discovering specific adaptation to these environments by detecting genes that are uniquely expressed in the natural environment (specific extremophile gene signature).

The evidence of existence of such a specific gene signature is being gathered on a gene-by-gene basis. For example, *Shewanella* genus is split into 2 major subgenuses: mesophilic pressure-sensitive species and high pressure-cold-adapted species, the latter shown to produce large amounts of eicosapentaenoic acid (Kato & Nogi 2001), which affects the membrane fluidity, shown to be an important component of pressure adaptation (MacDonald 1987). Microarray approach proposed here gives a more global perspective into a great number of genes affected by a change of a single parameter (such as pressure or temperature) and yield a better understanding of the changes required for specific adaptations of microorganisms living under such conditions (piezophiles, hyperthermophiles). A well defined hyperthermophile *M. jannaschii*, which genome has been sequenced (Bult et al. 1996) was chosen as a starting point in the series of the proposed experiments.

Additional insight into specific adaptation of hyperthermophiles could come through computer analysis of thus far sequenced genomes of hyperthermophiles and discovery of their gene signature. The necessity of such an approach is recognized throughout the field (Ng et al. 2000).

Moreover, the comparison of the genes of all available extremophile genomes sequences may reveal a group of common genes across these extremophile microorganisms called here general extremophile gene signature. Although the majority of extremophiles are confined to a specific extreme environment, some of them can thrive in more than a single extreme environment. The latter opens the possibility of existence of a general extremophile gene signature. An example supporting this view is Chroococcidiopsis species with its remarkable tolerance of environmental extremes: forms belonging to this species are present in a wide range of extreme environments: from Antarctic rocks to thermal springs and hypersaline habitats (Friedman & Ocampo-Friedman 1995).

Genetic engineering has been well established for cyanobacteria and the methods for insertion of clusters of genes developed (Billi et al. 2001). This opens a technical possibility of inserting a subset of genes of interest, for example pressure adaptation genes, into a mesophile (such as a cyanobacterium species) and testing their importance in survival under increased hydrostatic pressure. The concepts of the climate modeling (Meadows et al. 2001) will be applied towards development of hypothetical models of environments in space.

4. PRELIMINARY RESULTS

Our approach of extremophile genome comparisons was first developed for hyperthermophilic microorganisms, organisms which grow at 90°C or higher and have the highest growth temperatures known for life.

We have developed an initial software which incorporated features of Basic Local Alignment Search Tool (BLAST)[1] genome and BLAST protein and existing databases for several sequenced hyperthermophiles and were analysed using COGs (clusters of orthology) as a bases for comparisons.

The first step was exclusion of all genes present in bacteria which do not live in extreme enviroments. We used *E. coli K12*, a common laboratory strand, for these purposes. Therefore, the first step of comparisons was between *Pyrococcus abyssi (P. Abyssi)* (one of the 7 hyperthermophiles analysed) and *E. coli*, which eliminated all the common COGs and only remaining COGs (around 400) were used for search of the COGs common to hyperthermophiles.

Our analyses focused on 7 hyperthermophiles with well defined COGs available in the public domain. The 6 archaeal genomes were chosen to represent a wide variety of hyperthermal habitats. This approach significantly reduced the number of common genes which would be found among the Archaea more closely related, since our goal was the search for the minimal common genes to all hyperthermophiles. This goal was limited by the number of currently sequenced hyperthermophile genomes and the annotations of those which were sequenced.

[1] for more details see: http://www.ncbi.nlm.nih.gov/blast

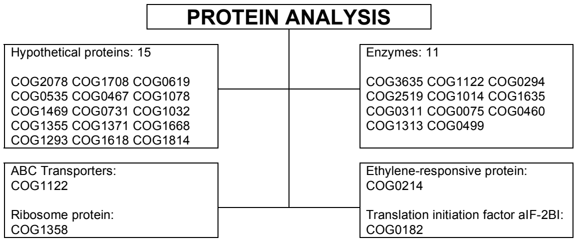

Fig. 1. *Hyperthermophile-specific protein groups. Proteins were grouped according to function of hypothetical proteins, enzymes, sensing systems (ABC transporters) and ethylene-responsive element and proteins involved in translation.*

The purpose of these analyses was the identification of genes involved in adaptation to extreme environment, in this case extreme temperatures (over 90°C) and extreme pressure. We identified 29 proteins common to all 7 hyperthermophiles which we grouped according to function into 4 categories (Fig. 1): hypothetical proteins (15 identified proteins had unknown function and were annotated as hypothetical), enzymes (11; see Table 1), membrane and ribosomal proteins (2) and regulators of gene expression (2).

Table 1. Enzymes common to 7 analysed hyperthermophiles. Enzymes include a rare enzyme which utilizes Tungsten as a co-factor: *COG0535*. There have been only 4 such enzymes discovered so far, but their importance in a tungsten-reach environment of deep-ocean hydrothermal vents is becoming more appreciated.

	Enzymes
COG3635	Phosphonopyruvate decarboxylase, putative
COG0294	DIHYDROPTEROATE SYNTHASE
COG2519	PROTEIN-L-ISOASPARTATE METHYLTRANSFERASE HOMOLOG
COG1014	2-ketoglutarate ferredoxinoxidoreductase, subunit gamma (korG-1)
COG0311	Imidazoleglycerol-phosphate synthase, subunit H, putative
COG0075	SERINE-GLYOXYLATE AMINOTRANSFERASE related (EC2.6.1.45) (SERINE-GLYOXYLATE AMINOTRANSFERASE)
COG0460	Homoserinedehydrogenase(hom)
COG0467	RECOMBINASE related
COG0535	Tungsten-containing aldehyde ferredoxin oxidoreductase COFACTOR MODIFYING PROTEIN
COG1313	Pyruvate formate-lyase activating enzyme (pflX)
COG0499	Adenosylhomocysteinase(ahcY)

At such extreme heat, as found in these environments, proteins are expected to loose their tertiary structure, denature, due to coagulation. The other challenge is sustaining plasma membrane in semi-liquid state. Yet another is the functioning of enzymes at these temperature. The categorizes of proteins we identified reflect these issues.

5. DISCUSSION AND FUTURE WORK

Comparative genomic analyses has the potential to generate hypotheses regarding the importance of specific genes and molecular characteristics for life in extremely cold environment, such as the permafrost (Ponder et al. 2003). The first psychrophile genome sequenced was *Psychrobacter strain 273-4*. It contains a 2.64 Mbp genome with 2, 147 ORFs. The approach of identification of cold adaptation genes involved comparison of 2 psychrophile genomes (*Psychrobacter 273-4* and *Exiguobacterium 255-15*) with the strains of these bacteria that live in warm waters and can grow at temperatures up to 42°C (Ponder et al. 2003). Using this approach, Ponder et al. (2003) identified one extremely large hypothetical protein (6,715 amino acids) and four histone-like proteins potentially involved in cold adaptation. However, comparing the genomes of 2 psychrophiles sequenced at that time, showed 75% of ORFs in Exiguobacterium encode for putative protein homologues in Psychrobacter. Our approach is taking advantage of a larger number of available sequenced genomes and will use comparisons of these genomes only and subtraction of genes found in a mesophile (*E. coli strain K12*), a method proven successful in hyperthermophile computational analyses (Sec. 4).

In the Solar system the variety of different conditions exists. These conditions depend on the distance of the objects from the Sun and surrounding planet(s) or chemical and physical characteristics of the object. Therefore, models designed to address possibility of existence of living forms in our solar system have to include and understand all factors involved.

From the analyses of comets (Meech et al. 2005; Jones et al. 2006), planets such as Mer-

cury, Mars, Venus or satellites around Earth (Moon), Jupiter, Saturn, Uranus and Neptune, a lot of different data regarding conditions of soil, atmosphere, temperatures, pressure etc. were collected. Our goal is to make a map of as many as possible areas of the objects in Solar system, and compare these with extreme conditions on the Earth. Example: the temperature in some areas on Mars, Callisto, Ganymede or Europa is similar to that on Antartica, but it is also very important to compare other components of extreme conditions (chemical and physical, such as pressure or existence of O_2 or metan).

We intent to analyse all collected data from the NASA and the others databases of planetary and Solar exploration, make our databases of conditions in extreme environments, and compare these databases. This will lead to construction of Atlas of planetary conditions potentially suitable for life and development of web application where conditions of an Earth extreme environment of interest could be compared to the closest resembling counterpart in space.

During the course of analyses some COGs were found to be common to only limited number of hyperthermophiles. For example, a *COG1361*, an S-layer protein representing a glycoprotein which is a cell wall component of some hyperthermophiles was common to *P. abyssi, Pyrococcus horikoshii (P. horikashii)* and *M. jannaschii*. This structure was previously implicated in survival of extreme temperatures. However, we didn't find to be present in any of 7 hyperthermophiles analysed and therefore not presented here. Likewise, many other genes which were common to subgroups of the analysed hyperthermophiles (but not to all of them,)were not the scope of this study.

6. CONCLUSIONS

This study revealed a large number of COGs common between *E. coli* and *P. abyssi* showing that such two distinct representatives of Archaea and Bacteria have a large portion of proteins in common.

We determined 29 COGs specific to only 7 studied hyperthermophiles and distinct from mesophiles exemplified in *E. coli*. We anticipated a smaller number of COGs to be found because the hyperthermophiles chosen were so diverse (including one aerobe and several strict anaerobes). Moreover, when a halophile (Halobacterium) was input in this program, no further restriction of the common genes was observed (data not shown).

However, since there is an extreme interest in these extremophile organisms, the sequencing of their genomes advances with amazing speed and the authors anticipate that the input of novel sequences (COGs) in our program will lead to further reduction of the genes common to hyperthermophiles.

REFERENCES

Bult, C. J., White, O., Olsen, G. J., Zhou, L., Fleischmann, R. D., Sutton, G. G., Blake, J. A., FitzGerald, L. M., Clayton, R. A., Gocayne, J. D., Kerlavage, A. R., Dougherty, B. A., Tomb, J. F., Adams, M. D., Reich, C. I., Overbeek, R., Kirkness, E. F., Weinstock, K. G., Merrick, J. M., Glodek, A., Scott, J. L., Geoghagen, N. S., & Venter, J. C., 1996, Science, 273, 1058

Cavicchioli, R., Thomas, T., & Curmi, P. M., 2000, Extremophiles, 4, 321

Cavicchioli, R., 2002, Astrobiology, 2, 281

Christner, B. C., Mosley-Thompson, E., Thompson, L. G., & Reeve, J. N., 2001, Environ. Microbiol., 3, 570

D'Amico, S., Claverie, P., Collins, T., Georlette, D., Gratia, E., Hoyoux, A., Meuwis, M. A., Feller, G., & Gerday, C., 2002, Philos. Trans. R. Soc. Lond. B. Biol. Sci., 357, 917

Feller, G., & Gerday, C., 2003, Nat. Rev. Microbiol., 1, 200

Friedmann, E. I., & Ocampo-Friedmann, R., 1995, Adv. Space. Res., 15, 243

Goodchild, A., Raftery, M., Saunders, N. F., Guilhaus, M., & Cavicchioli, R., 2004, J. Proteome. Res., 3, 1164

Hearn, E. M., Dennis, J. J., Gray, M. R., & Foght, J. M., 2003, J. Bacteriol., 185, 6233

Jones P.A., Burton M.G., Sarkissian1 J.M., Voronkov M.A., Filipović M. D., 2006, MNRAS, 369, 1995

Kato, C., & Nogi, Y., 2001, FEMS Microbiol. Ecol., 35, 223

MacDonald, A., 1987, In: M. R. Jannasch HW, Zimmerman AM (ed.), High pressure biology, London: Academic Press, pp. 207,

Margesin, R., & Schinner, F., 1999, Chemosphere, 38, 3463

Meadows, V., 2005, In: Modeling the diversity of extrasolar planets, Proceedings of the International Astronomical Union, 1, 25

Meech K.J., et al., Filipović M.D., et al., Deep Impact: Observations from a Worldwide Earth-Based Campaign, 2005, Science, 310, 265

Navarro-Gonzalez, R., Rainey, F. A., Molina, P., Bagaley, D. R., Hollen, B. J., de la Rosa, J., Small, A. M., Quinn, R. C., Grunthaner, F. J., Caceres, L., Gomez-Silva, B., & McKay, C. P., 2003, Science, 302, 1018

Ng, W. V., Kennedy, S. P., Mahairas, G. G., Berquist, B., Pan, M., Shukla, H. D., Lasky,

S. R., Baliga, N. S., Thorsson, V., Sbrogna, J., Swartzell, S., Weir, D., Hall, J., Dahl, T. A., Welti, R., Goo, Y. A., Leithauser, B., Keller, K., Cruz, R., Danson, M. J., Hough, D. W., Maddocks, D. G., Jablonski, P. E., Krebs, M. P., Angevine, C. M., Dale, H., Isenbarger, T. A., Peck, R. F., Pohlschroder, M., Spudich, J. L., Jung, K. W., Alam, M., Freitas, T., Hou, S., Daniels, C. J., Dennis, P. P., Omer, A. D., Ebhardt, H., Lowe, T. M., Liang, P., Riley, M., Hood, L., & DasSarma, S., 2000, Proc. Natl. Acad. Sci. USA, 97, 12176

Onstott, T. C., McGown, D., Kessler, J., Lollar, B. S., Lehmann, K. K., & Clifford, S. M., 2006, Astrobiology, 6, 377

Ponder, M. A., Gilmour, S. J., Bergholz, P. W., Mindock, C. A., Hollingsworth, R., Thomashow, M. F., & Tiedje, J. M., 2005, FEMS Microbiol. Ecol., 53, 103

Price, P. B., & Sowers, T., 2004, Proc. Natl. Acad. Sci. USA, 101, 4631

Price, P. B., 2007, FEMS Microbiol. Ecol., 59, 217

Saunders, N. F., Thomas, T., Curmi, P. M., Mattick, J. S., Kuczek, E., Slade, R., Davis, J., Franzmann, P. D., Boone, D., Rusterholtz, K., Feldman, R., Gates, C., Bench, S., Sowers, K., Kadner, K., Aerts, A., Dehal, P., Detter, C., Glavina, T., Lucas, S., Richardson, P., Larimer, F., Hauser, L., Land, M., & Cavicchioli, R., 2003, Genome. Res., 13, 1580

Siddiqui, K. S., & Cavicchioli, R., 2006, Annu. Rev. Biochem., 75, 403

Skidmore, M., Anderson, S. P., Sharp, M., Foght, J., & Lanoil, B. D., 2005, Appl. Environ. Microbiol., 71, 6986

Skidmore, M. L., Foght, J. M., & Sharp, M. J., 2000, Appl. Environ. Microbiol., 66, 3214

Tung, H. C., Bramall, N. E., & Price, P. B., 2005, Proc. Natl. Acad. Sci. USA, 102, 18292

ПРИМЕРИ МОЛЕКУЛАРНЕ АДАПТАЦИЈЕ НА ЕКСТРЕМНЕ УСЛОВЕ ЖИВОТНЕ СРЕДИНЕ И ПРИМЕНА НА СВЕМИРСКУ ОКОЛИНУ

M. Filipović[1], S. Ognjanović[2] and M. Ognjanović[2]

[1] *University of Western Sydney, Locked Bag 1797, Penrith South DC, NSW 1797, Australia*

[2] *University of Minnesota, MMC 715, 1-185 Moos*
420 Delaware Street SE Minneapolis, MN 55455, USA

Представљамо иницијална истраживања структура гена одговорних за адаптацију микроскопског живота у екстремним условима на Земљи. Овде, прелиминарно презентујемо резултате идентификације клустера ортхологус гроупа (COGs) заједничких за неколико хипертермофила и изузимање оних заједничких за мезофиле (не-хипертермофиле): *E. coli K12*, скупљени у групу могућих протеина одговорних за адаптацију живота у екстремним условима. Компаративна генетичка анализа представља снажно оруђе у откривању нових гена одговорних за адаптацију у екстремним условима. Метаногени представљају једину групу организама који могу да 'расту' на 0°C (*M. frigidum* и *M. burtonii*) и 110°C (*M. kandleri*). Мада, не све термичке компоненте адаптације се могу приписати тим новим генима, 'chaperones' познатији као топлотни удар протеин стабилизује ензиме при повећању температуре. Наш циљ је коришћење специјално развијеног софтвера за компаративну анализу гена значајних за адаптацију хипертермофила. Следећи хипертермофилски гени су уврштени у софтвер за потребе ове студије: *M. jannaschii, M. kandleri, A. fulgidus* као и три врсте *Pyrococcus*. Заједнички гени, лоцирани су и груписани према њиховој улози у ћелијским процесима. Додатни експериментални податци су неопходни за даље изучавање ових протеина.